Evaluation of Antibacterial Activity and Interaction of Methanolic Extract of *Physcia grisea* and Cephalosporin on Clinical Isolates of *Streptococcus pyogenes*

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Abstract

This study was carried out to investigate the antibacterial activity and interaction of methanolic extract of Physcia grisea and cephalosporin on the clinical isolates of Streptococcus pyogenes. The isolates of S. pyogenes used were obtained from the department of Pharmaceutical microbiology, University of Nigeria, Nsukka. The sensitivity pattern of these isolates to P. grisea extract, cephalosporin and P. grisea extract combined with cephalosporin was carried out using agar diffusion technique. The result of the in vitro study showed that the MIC of P. grisea extract was decreased by cephalosporin thereby increasing its efficacy. The test micro-organism, S. pyogenes was highly sensitive to P. grisea combined with cephalosporin, but moderately sensitive to P. grisea extract. This shows that in the treatment of streptococcal infections caused by S. pyogenes, combined P. grisea, extract and cephalosporin may give the best therapeutic effect.

Keywords: Antibacterial activity, Interaction, Methanolic Extract, *Physcia grisea,* Cephalosporin, *Streptococcus pyogenes*

Introduction

Herbal medicinal products are becoming more widely accepted as alternatives to medical prescriptions (Gaedcke and Steinhoff, 2003). These herbal medicines are complex mixtures of more than one active ingredient. This multitude of active ingredients increases the possibilities of interactions between herbal medicines and conventional (synthetic) drugs (Ernst, 2000). Thus, extract from *Physcia grisea* that possesses a broad spectrum of antimicrobial actions (Eze and Ogonnaya, 2010) may have a desired interaction for therapeutic use. *P. grisea* is lichen from phylum of thalophyta with abundant antimicrobial substances and are thus worthy of further studies (Eze *et al*, 2009).

P. grisea is found on walls, rocks, and trees attached by short threads which grow from the under side and are white with black tips. The plant is light grey or slightly brownish grey, and is almost always covered, at least near the tips of the lobes with a very fine white powder. The colour develops a greenish tinge when the plant is wetted (Nicholson, 1966). *S. pyogenes* is a Gram positive non motile bacterium that causes acute sore throat, scarlet fever, ear infections, puerperal sepsis and skin infection such as cellulites, septicaemia and occasionally endocarditis (Cheesbrough, 1985).

The common drugs for the treatment of infections caused by *S pyogenes* have been cephalosporin, penicillin and erythromycin despite their toxicity and other short comings. There is therefore, a need to investigate on medicinal plant extracts that will be safe, stable and selectively toxic to this organism that causes these streptococcal infections. This study was aimed at evaluating the antibacterial activity of *P. grisea* extract and the *in vitro* interaction between *P. grisea* extract and cephalosporin on *S. pyogenes*.

Materials and Methods

The test microorganism used for this research was clinical isolates of *S. pyogenes* obtained from the Department of Pharmaceutical Microbiology, University of Nigeria, Nsukka. The drug for the treatment of *S. pyogenes* infections was from Fidson Healthcare, Nigeria Plc and the culture media used were nutrient agar and nutrient broth (Oxoid).

Sources of samples: The *P. grisea* used for this work was obtained from the back of *Dialum guinese* tree in Ezimo, Udenu Local Government Area of Enugu State, Nigeria. The *P. grisea* was identified in the Department of Botany, University of Nigeria, Nsukka.

Method of extraction: The method of Eze *et al* (2010) was used in the extraction of *P. grisea* used. About 14.0 g of pulverized *P. grisea* materials were weighed out using mettler sensitive balance and poured into 500 ml flat bottom flask. This was soaked in 250 ml of absolute methanol to get 56 mg/ml. the content of the flask was stirred with magnetic stirrer for 18 hours and then left to stand for 24 hours before it was filtered using a clean muslin cloth. The filtrate was concentrated in the oven (Gallen Kamp, England) at 60 ^{0}C.

Sensitivity testing: The sensitivity testing of cephalosporin, *P. grisea* extract both in a single and in a combined dose, were evaluated by the agar diffusion method as described by Agboke *et al*, (2005) to determine the inhibition zone diameter (IZD) of the agents as well as sensitivity pattern of the clinical isolates of *S. pyogenes*.

Determination of the IZD of the extract on *S. pyogenes:* Sterile Petri dishes were aseptically seeded with 0.1 ml of freshly prepared suspension of *S. pyogenes* using a sterile pipette. A 20 ml aliquot of a sterile molten nutrient agar at 45 ^{0}C in McCartney bottle was poured into each plate and swirled clockwise and anti-clockwise for even distribution of the organism. After solidifying, the agar plates were marked into four sections representing the four two-fold dilution of the extract (200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml) and labelled 1 – 4 with an indelible marker. Using a sterile 6mm cork-borer, cups were made in each of the four divisions. The two fold dilutions of the *P. grisea* extracts were aseptically added in the cups using standard sterile dropper starting with the highest concentration of the *P. grisea* extract to that with lowest concentration of the extract.

The plates were incubated at 37 ^{0}C for 24 hrs and then, the zones of inhibition were measured. This was repeated and the average values of the zones of inhibition were determined.

The graph of the inhibition zone diameter square against the logarithm of the concentrations of the dilutions used was plotted and the MIC of the extract was then determined from the graph.

Determination of IZD of cephalosporin: This was determined as described above except that the extract was replaced with 2.5 mg/ml, 1.25 mg/ml, 0.63 mg/ml, and 0.31 mg/ml of cephalosporin solutions.

Determination of IZD of extract and cephalosporin: This was carried out as described above only that the solutions of the extract and the drug were combined (202.5 mg/ml, 101.3 mg/ml, 50.6 mg/ml and 26.3 mg/ml) and used on *S. pyogenes*.

Results

The sensitivity *S. pyogenes* to *P. grisea* both in single and in combined dose showed that *S. pyogenes* was highly sensitive to combined cephalosporin and the extract but moderately sensitive to extract and cephalosporin in single dose (Table 1).

Table 1: Sensitivity of *S. pyogenes* to antimicrobial agents

Antimicrobial agents	*S. pyogenes*
***P. grisea* extract**	++
Cephalosporin	++
Extract and cephalosporin	+++

++ = S. pyogenes was moderately sensitive to P. grisea extract and cephalosporin in single dose +++ = S. pyogenes was highly sensitive to combined P. grisea extract and cephalosporin

The MIC of *P. grisea* extract was 6.31 mg/ml while that of *P. grisea* extract combined with cephalosporin was 4.47 mg/ml. The MIC of the cephalosporin only was 0.25 (Tables 2, 3 and 4).

Table 2: Table of mean values of concentration, log concentration, 1ZD and 1ZD^2 of *P. grisea* extract on *S. pyogenes*

Concentration (mg/ml)	Log Conc.	1ZD (mm)	IZD^2 mm^2)	MIC (mg/ml)
200	2.30	17	289	6.31
100	2.00	15	225	
50	1.60	13	169	
25	1.40	12	144	

Values are mean of replicates from three trails after 24 hours of incubation

Table 3: Table of mean values of concentration, log concentration, 1ZD and 1ZD^2 of cephalosporin *S. pyogenes*

Concentration (mg/ml)	Log Conc.	1ZD (mm)	IZD^2 (mm^2)	MIC (mg/ml)
2.5	0.4	26.0	676	0.25
1.25	0.1	22.0	484	
0.63	-0.2	17.0	289	
0.31	-0.5	14.0	196	

Values are mean of replicates from three trails after 24 hours of incubation

Table 4: Table of mean values of concentration, log concentration, 1ZD and 1ZD^2 of *P. grisea* and cephalosporin *S. pyogenes*

Concentration (mg/ml)	Log Conc.	1ZD (mm)	IZD^2 (mm^2)	MIC (mg/ml)
202.5	2.31	27	729	4.47
101.3	2.01	23	529	
50.6	1.70	19	361	
25.3	1.40	16	256	

Values are mean of replicates from three trails after 24 hours of incubation

Discussion

At present, incidences of streptococcal infections caused by some resistant species of streptococcus have been reported in medical practice (Cheesbrough, 1985). The challenge has been to develop effective therapeutic measure for streptococcal infections, considering the increase in the development resistance by *S. pyogenes* to synthetic drugs or antibiotics. One way of achieving this may be to administer a safe and stable natural antibacterial agent from *P. grisea* with broad spectrum of activity either singly or concurrently.

In this study, the administration of *P. grisea* extract in single dose showed that it has moderate antibacterial activity on *S. pyogenes*. This means that *P. grisea* could represent a led source of novel antimicrobial drugs (Eze and Ogonnaya, 2010) if properly utilized. In rational drug therapy, the concurrent administration of two or more antimicrobial agents is often essential and sometimes mandatory in order to achieve the desired therapeutic effect (Attama *et al*, 2005 and Aguwa, 1996). This was why the *P. grisea* extract was combined with another antimicrobial agent like cephalosporin in this research. The result of the interaction of the two agents showed that cephalosporin decreased the minimum inhibitory concentration (MIC) of the *P. grisea* extract from 6.31 mg/ml to 4.47 mg/ml. This reduction in MIC of

the combined *P. grisea* and cephalosporin is in line with Ofokansi and Esimone (2005). Their findings showed that the application of lichen extract (*Ramalina farinacea*) was generally better, in terms of rapidity of action when combined with synthetic/standard drugs. The combination of these agents could lead to extension of the antimicrobial (antibacterial and antifungal) spectrum because it is possible that two or more infections microorganisms with different sensitivity patterns have to be dealt with (Agboke *et al*, 2005). This is necessary in this case since *S. pyogenes* infections are usually associated with other bacterial and fungal infections. Besides, treatment with *P. grisea* extract in streptococcal infections can help to maintain or restore balance of the normal flora since it has both antifungal and antibacterial properties (Eze, 2007).

It is therefore recommended that the clinical significance of this interaction has to be determined for suitable application since the combination may be good enough to treat infections caused by *S. pyogenes*.

References

Agboke, A. A., Eze, E. I. and Adikwu, M. U. (2005) Combined activities of colloidal silver concentration and cephalexin on *Staphylococcus aureus* using the agar diffusion technique; *Bio-Research,* 3(2): 7 – 10.

Aguwa, C. N. (1996). Drug Interaction. *In: Therapeutic Basis of Clinical Pharmacy in the Tropics*, 2nd Edition; Aguwa, C. N. (Ed); Enugu Optimal Publishers, 148 – 150.

Attama, A. A., Nnamani, P. O. and Nwafor, C. N. (2005). *In vitro* study of the interaction between amoxicillin and norfloxacin with lansoprazole; *Bio-Research,* 3(2); 52 – 55

Cheesbrough, M. (1985). *Medical Laboratory Manual for Tropical Countries.* Volume II, Microbiology, Butterworth and Company Publisher Limited, London.

Ernst, E. (2000). Interactions between synthetic and herbal medicinal products. Part 2, A systematic review of the direct evidence. *Perfusion,* 13: 4 – 15

Eze, E. I. (2007). *Sensitivity pattern of clinical isolates of Candida albicans and Escherichia coli from HIV/AIDS patients in Nsukka area of Enugu State to Combined Physica grisea extract and standard antimicrobial agent*. Nigerian Institute of Science Laboratory Technology Fellowship Thesis.

Eze, E. I., Ezeugwu, C. N. and Adikwu, M. U. (2009). Sensitivity pattern of clinical isolates of *Candida albicans* from HIV/AIDS patients to combined *Physcia grisea* extract and tioconazole. *Global Journal of Pure and Applied sciences,* 15(3 & 4): 301 – 304.

Eze, E. I., Onu, E. O., Iwueze, C. A. and Ugwu, G. C. (2010). Antimicrobial activities of methanolic extract of *Gongronema latifolia* stem on clinical isolate of *Escherichia coli* from diarrhea patients. *Global Journal of Pure and Applied Science,* 16(4): 391 – 394.

Eze, E. I. and Ogonnaya, F. N (2010). *In–vitro* evaluation of antimicrobial activity of ointment containing *Physcia grisea* extract on *Candida albicans*. *Animal Research International,* 7(3): 1253 – 1256.

Gaedcke, F. and Steinhoff, B. (2003). *Herbal Medicinal Products: Scientific and Regulatory Basis for Development, Quality Assurance and Marketing Authorization*. Medpharm Publisher, London.

Ofokansi, K. C. and Esimone, C. O. (2005). Evaluation of *in vitro* antimicrobial activity and release behaviour of ointments and applications containing extracts of the lichen *Ramalina farinaceal* (L) ach. *Plant Product Research Journal,* 9: 6 -10

Nicholson, B. E. (1966). *The Oxford Book of Flowerless Plants Ferns, Fungi, Mosses and Liverworts, Lichens, and Seaweeds*. Oxford University Press, Oxford.